Application No. 09/920,435
Filed: August 1, 2001
Group Art Unit: 1639

REMARKS

Claims 1-22 are pending in the present application. Claims 15-20 are withdrawn from consideration due to a restriction requirement. Claims 3 and 13 are amended herein. Accordingly, claims 1-14, 21 and 22 will be pending upon entry of the instant amendments and claims 15-20 are withdrawn from consideration.

Support for the amended claims can be found throughout the specification and encompassed by the scope of the claims as originally filed. The amendments to the claims are further explained below. No new matter has been added.

Any amendments to the claims should in no way be construed as acquiescence to any of the Examiner's rejections and was done solely to expedite the prosecution of the application. Applicants reserve the right to pursue the claims as originally filed in this or a separate application(s).

Claim Rejections - 35 U.S.C. §112

Claims 1-14 and 21-22 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite.

Applicants respectfully traverse the rejection.

Claims 1 and 14 are considered indefinite and/or unclear due to the use of the term "natural." One of ordinary skill in the art would be able to determine the scope of what "natural" sample means with respect to the present application. The present specification clearly describes what is encompassed by the term "natural." On page 2 of the specification, first full paragraph, natural samples encompass those that are highly and chemically diverse collection of compounds that include very small to very large molecules, which makes it difficult to isolate any single active compound. Exemplary natural samples in accordance with the

invention comprises those samples listed on page 8 of the specification, last full paragraph, which have been collected in their natural status and can be natural products, samples, or extracts. One of ordinary skill in the art of the invention would understand that "natural" sample refers to a naturally occurring biological material, such as plant matter, animal matter, extracts from such materials, and the like. In contrast, samples other than natural samples distinguished in the specification are combinatorial libraries, which are made synthetically containing a large number of different chemicals. An ordinary skilled artisan would know the starting material of a given analysis considering that this a method of targeting and obtaining ligands found in plants and organisms found naturally. Therefore, Applicants respectfully request reconsideration of this rejection.

Claim 3 is considered indefinite for the phrase "having a molecular weight of about 1,500 or less." Claim 3 has been amended herein to recite "a molecular weight of about 1,500 daltons or less" as suggested by the Examiner.

Claim 13 is considered indefinite because the claims that this is dependent on do not refer to "step (6)." Applicants amended claim 13 to obviate this concern.

Claim 14 is considered indefinite because, according to the Examiner, "it is not clear how subjecting either a sample of the protein target alone or a mixture of the protein target with a non-target-binding natural sample could function as a reference standard because it is not clear what is being referenced."

To explain, the method step in claim 14 is a way to provide a reference standard for determining the qualitative and quantitative presence of a ligand of interest discovered in a complex natural sample. For example, by having the analytical

results of the protein target alone with a protein target in combination with a natural ligand sample, an ordinary skilled artisan can compare and determine if there is a presence of a natural ligand that binds with the protein target. A standard having a mixture of the protein target with a non-target-binding natural sample can provide information on any anomalies occurring in the run. The ultimate end result would provide information on isolating the ligand found in the natural sample that binds to the protein target of interest. Applicants consider that claim 14 is clear and definite. Accordingly, Applicants respectfully request reconsideration and withdrawal of the foregoing rejection.

Claim Rejections - 35 U.S.C. §102

Claims 1-14 and 21-22 are rejected under 35 U.S.C. §102(e) as being anticipated by Nash et al. (U.S. Patent 6,207,861). The Examiner states that "Nash et al. discloses a method for identifying members of a mass-coded combinatorial library which are ligands for a biomolecule wherein said biomolecule can be a protein that anticipates claim 1."

Applicants respectfully traverse the foregoing rejection.

The claimed invention is directed to a method of screening a complex natural sample for an affinity ligand that binds to a protein target, where, initially, a protein target and a natural sample is mixed in solution. Immediately, one can distinguish the cited art from the present application. Nash et al. is directed to a method of producing a mass-coded set of chemical compounds to be used as a screening tool for identifying a ligand to a particular biomolecule. In contrast, the presently claimed invention comprises screening complex natural samples for ligands that bind to protein targets of interest. As explained above, the

complex natural sample is distinguishable from any synthetic combinatorial library due to the difficulty in isolating a ligand from a complex natural sample. The cited art does not address the special challenges presented by screening natural samples, which typically contain a wide range of biomolecules of varying size or molecular weight. The prior art focuses on screening combinatorial, small molecule libraries rather than natural samples. Nash et al. applies a single filtering step that removes unbound small molecules, such as, using a gel filtration. Therefore, Nash et al. was intent only on separating out unbound, small ligands from larger complexes of small ligands bound to target, which were then subjected to mass spectrometry.

Additionally, Nash et al. fails to anticipate the recited method steps used in accordance with the invention. Nash does not provide the specific starting material nor the conditions required for practicing the method of the invention. In the method used for the development of ligands in Nash et al., the target protein is covalently biotinylated and immobilized by binding to a streptavidin-derived water-insoluble column matrix. The mass coded combinatorial library is then solubilized in a binding buffer and injected onto a column containing the protein-streptavidin complex. In contrast, the method in the present invention is simpler and can find potential ligands from a complex natural sample found in nature, in contrast to a combinatorial library. An extract of the natural sample is mixed in solution with a protein target and allowed to incubate until equilibrium. Then an aliquot of this mixture is injected into a first size-exclusion medium that removes any small molecular weight compounds each having a molecular weight less than a first preset value.

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Nash et al. also does not teach, nor anticipate, removing larger molecular weight compounds, including the target when it is large, prior to mass spectrometry. The cited art fails to teach or even suggest the need to apply another size-exclusion step (Applicants' step (5), e.g., ultrafiltration), to remove large molecules also present in the natural sample and the target. Even less does the prior art teach or suggest combining two size-exclusion steps as in Applicant's claimed screening method: i.e., removing small molecular weight compounds first (Applicant's step (3)) and then removing larger molecules prior to mass spectrometry (Applicants' step (5)). The cited art also fails to anticipate the particular dimensions of the size-exclusion media used by Applicants (e.g., size exclusion gel filtration or HPLC column, and ultrafiltration membrane), as recited in the dependent claims. For instance, Applicants' second size-exclusion medium excludes molecules having a molecular weight of at least 3000 Daltons or more (claim 11), preferably 10,000 Daltons or more (claim 10).

Not only does Applicants' present method enable screening of natural samples, it enables high throughput, automated screening of such material, and allows the process to proceed for longer periods of time before conduits in the screening apparatus, such as transfer lines, need be changed (see, e.g., specification, page 4, lines 2-21).

Based on the foregoing, Applicants consider that Nash et al. fails to anticipate each and every element of the claimed invention. Accordingly, reconsideration and withdrawal of the foregoing rejection are respectfully requested.

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Claim Rejections - 35 U.S.C. §103

Claims 1-14 and 21-22 are rejected under 35 U.S.C. §103(a) as being obvious over Kaur et al. (*J Prot Chem* 1997, 16(5):505-511) in view of Van Breeman et al. (*Anal Chem* 1997, 69:2156-2164).

Applicants respectfully traverse the rejection.

As described above, the method of the invention is directed to finding potential ligands found in natural samples. In the past, it has been difficult to isolate ligands found in natural samples due to the complexity of the sample. The present method of screening natural samples for ligands is an efficient simplified method of first finding a potential ligand and then characterizing that ligand using further analysis. The two primary components to the method of the invention include removing any small molecules that are not bound by a ligand using a first size exclusion medium and after dissociation of the ligand from the target protein, removing any large molecules including any large target proteins using a second size-exclusion medium. This is done to provide further analysis capability for the characterization of the ligand found in the natural sample using a simple one-process procedure. Additionally, the starting materials are mixed in solution to equilibrium to allow for complex formation by the target and any target binding ligand present in the natural sample.

Kaur et al. describes a method of identifying ligands by screening libraries of small molecules created by combinatorial synthetic methods. The method used for identifying lead compounds in combinatorial libraries involves affinity selection and mass spectrometry. Kaur et al.'s method only allows binding of compounds with the highest affinities to bind to the receptor by using receptor-limiting incubation conditions. While the unbound

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ligands are eluted off, an aliquot of the receptor/ligand complex is introduced into the ion source of an electrospray triple-quadrupole mass spectrometer and the complex is dissociated by desalting and concentrating on-line in an injector loop, using a peptide reversed-phase cartridge. Kaur et al. is distinguishable from the present invention such that its teaching or suggestion cannot make the present invention obvious.

In contrast to Kaur et al., the present invention provides certain method steps that allow for further isolation and characterization of a ligand found in complex natural samples. One of ordinary skill in the art would appreciate that it is not obvious to isolate ligands from complex natural samples based on the teachings of Kaur et al. since the cited art merely describes screening combinatorial libraries. No suggestion or teaching is recognized in Kaur et al., expressly or impliedly, to come up with the presently claimed invention nor is there any explanation to resolve matters in complex natural samples. In addition, as previously stated, the method of the present invention has two major steps. The ligand-target complex moves through two different size-exclusion mediums to specifically isolate the ligand of interest. As the Examiner stated, Kaur et al. fails to teach or suggest using a second size exclusion medium to remove large molecules, both from the natural sample and target, after dissociation of the ligand of interest.

Van Breemen et al. fails to cure the deficiencies found in Kaur et al. Van Breemen et al. also fails to recognize the difficulty of obtaining ligands from complex natural samples. Van Breemen et al. uses a pulsed ultrafiltration mass spectrometry for screening combinatorial libraries for compounds that bind to macromolecular receptors. Van Breemen et al.'s protein targets

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are trapped in an ultrafiltration membrane and then in a pulse, the combinatory library is passed through the ultrafiltration membrane. In contrast, the novel simplified method of the invention initially combines both the protein target and complex natural sample in a solution to allow for appropriate binding until equilibrium is reached. Then an aliquot of the mixture is then passed through a first size-exclusion medium. Moreover, Van Breemen et al. fails to teach or suggest providing two different size-exclusion mediums for the efficient isolation of the ligand of interest. Van Breemen et al., either alone or in combination, fails to teach or suggest the claimed invention.

Applicants respectfully request reconsideration and withdrawal of the foregoing rejection.

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CONCLUSION

Based on the foregoing, entry of the amendments and remarks presented herein, reconsideration and withdrawal of all the rejections and allowance of application with all pending claims are respectfully requested.

The Examiner is encouraged to telephone the undersigned attorney to discuss any matter that would expedite allowance of the present application.

Respectfully submitted,

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